Is chemotherapy-resistance in Triple-negative breast cancer lines associated with mesenchymal and epithelial traits?

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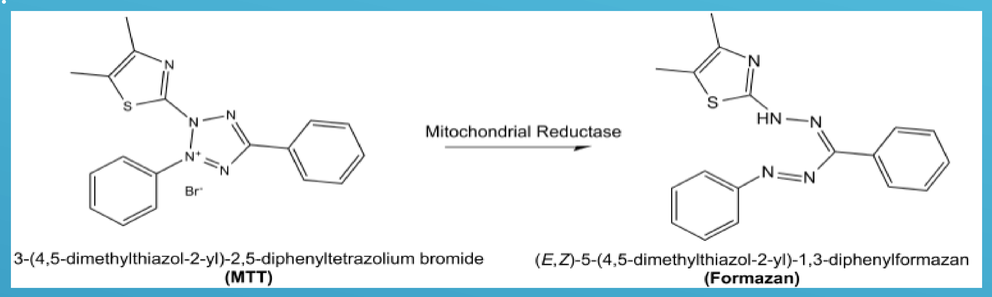
Introduction and Overview

The MTT assay is a colorimetric assay used for analyzing cell metabolic activity and cell viability as a function of redox potential. The MTT is absorbed actively by the cells that are being assessed causing MTT to be reduced via a mitochondrial-dependant reaction to generate a formazan product.



3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide **(MTT)**

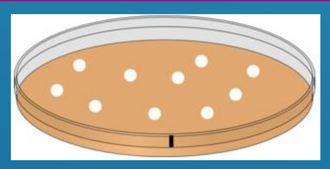
(*E*,*Z*)-5-(4,5-dimethylthiazol-2-yl)-1,3-diphenylformazan **(Formazan)**

The product, formazan, appears purple colored in living cells. Formazan product accumulates within the cell as it is unable to penetrate the cell membrane. As a result, Dimethyl sulfoxide (DMSO) is added to render formazan soluble. The capacity of the cells to reduce MTT provides an indication of mitochondrial integrity as well as its activity, which is used as a method in determining cell viability. This method was used to analyze MDA-MB231 breast cancer cell line to measure cell growth in culture and the levels of mitochondrial activity. The cell culture plate was incubated for 3 hours in culture medium in a 24-well plate. After the incubation period, the culture medium was removed via vacuum aspiration. 600uL of DMSO was added into each well. After a 30 minute wait, a 96-well plate was used to analyze the data. The absorbance of this colored solution can be quantified by measuring the 96-well plate at different wavelengths of 560nm, 570nm, and 590nm by a spectrophotometer. Yes-associated protein, YAP, is associated with cancer growth. On the other hand, Cabinostatin and Verteporfin are inhibitors of YAP and their inhibitory effects on YAP will be analyzed using the MTT assay. As a result, the degree to which Cabinostatin and Verteporfin inhibit YAP can provide an indication to the degree of tumorigenesis inhibition. In this experiment, DMSO will be used as the control.

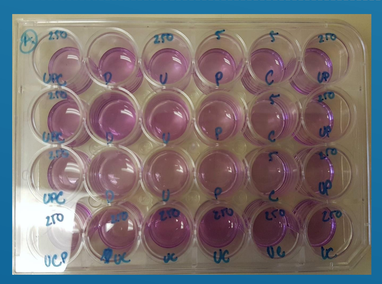
Objective

Determine the viability of the MDA-MB231 breast cancer cell line with controllable expression of different protein controls. The extent by which different protein expression affects were examined: (i) resistance to some chemotherapeutic drugs by determine cell viability (MTT or Almar Blue assay); (ii) chemotherapy-induced cancer stem cells based on expression of CD44 and/or ALDH marker determined by quantitative PCR or by flow cytometry.

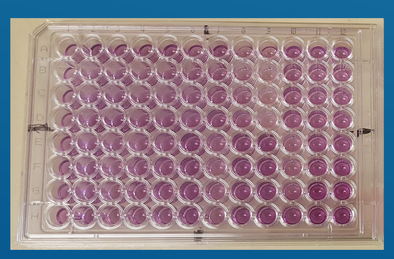
Methodology



1- Treat MDA-MB231 breast cancer cell line .



2- Run an MTT assay using a 24-well plate



3- Measure the absorbancance at 560nm ,570nm ,and 590nm using a spectrophotometer using a 96-well plate.

Results

YAP, Yes-associated protein, regulates a diversity of cellular process during development, but the most important trait that YAP contains which is targeted in this experiment is the fact that it plays an important role in tumorigenesis. Multiple drugs are used to counteract this trait, by inhibiting YAP, in order to find a way to slow or inhibit the growth of tumors. Figure 1 displays MTT viability relative to the control which is DMSO (additive of the drugs). The y-axis represents the inhibitory capability of drugs towards proliferation and it is relative to the control which is DMSO. Based on the results obtained, it is observed that Cabinostatin's inhibitory effects on YAP production is significantly lower when compared to the inhibitory effects of Verteporfin, a Porphyrin derivative. On the other hand, when Cabinostatin and verteporfin are used together, the inhibitory effect on YAP production is even much lower.

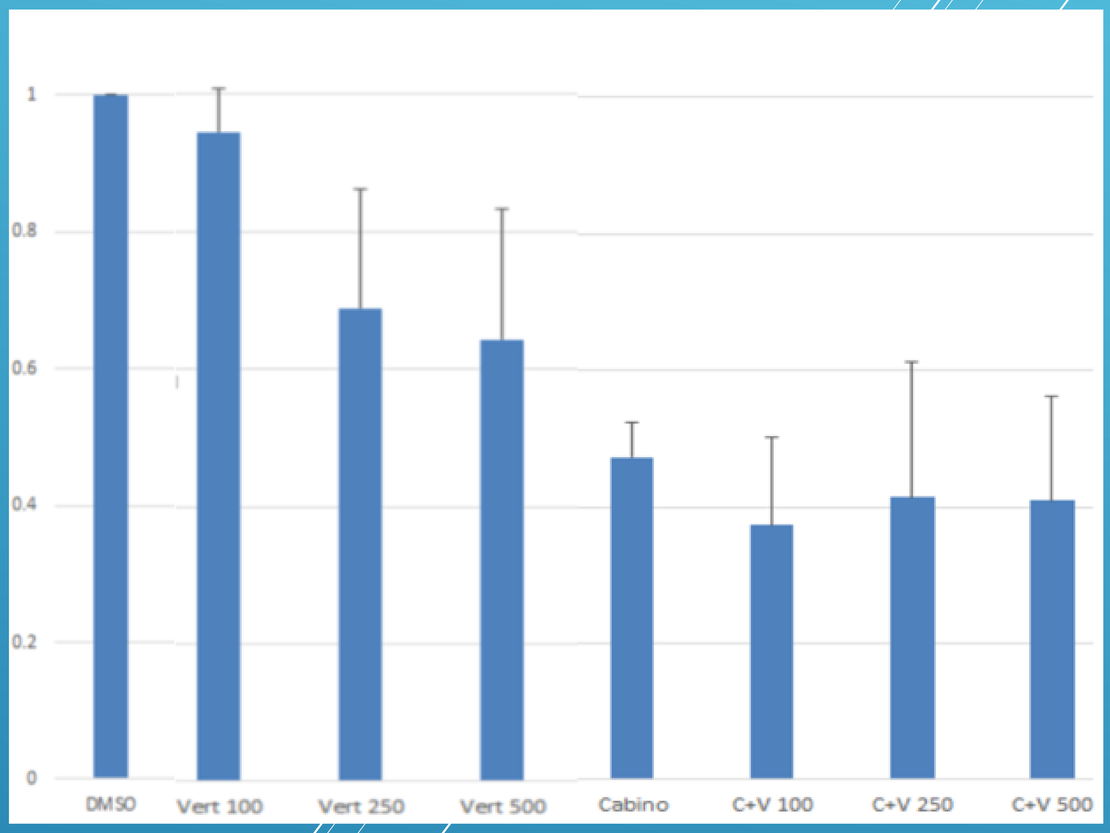


Figure 1.

Conclusion

Verteporfin's inhibitory effects on tumorigenesis is much more significant compared to the inhibitory effects of cabinostatin. In addition, when verteporfin is used separately, it produces a stronger inhibitory effect than when it is combined with cabinostatin.

Future Work

Identifying how chemotherapy and chemotherapeutic drugs change the protein expression of triple negative breast cancer to promote resistance with the goal of identifying potential new targets.

Acknowledgement

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